(1)

June 21, 1950

Dear Josh,

Here comes letter number 3 with detailed comments. I've shipped your first to Ryan in SA (if you want his schedule to September just let me know). Concerning your second letter first:

(a) Modification of the thesis is possible - I haven't deposited a typewritten copy yet (which has to be submitted in lieu of published copies until such time as printed copies are obtained).

(b) FJ will be reasonable but reasonable by his own standard of reasonableness.

(c) Shaking the tubes in a cross probably increases collisions (see detailed comments below). 5300 collision efficiency is low (high efficiency, low value), some crosses run 20 to 50 K.

(d) Agglutination probably does occur, at least there are local concentrations of bacteria in culture tubes (that is, centrifuge tubes in which the cross is being run). If clumping occurs how is the Poisson distribution to be explained (Table 2)? Of course the zero order kinetics may really be an antitangent function, probability integral, required to describe the aggregation of a minimum number of cells before syngamy-transformation (synformation). This math treatment would be similar to multihit (and really multi) killing curves.

To isolate the zygote - if only phage resistance were dominant to phage sensitivity - cross a resistant/sensitive X sensitive/resistant and after an hour's mix add the two types of phage. What about drug resistance? The same method could be applied here using two markers if the dominance relationships are right except of course the non-active cells (not undergoing syngamy) would not be affected unless the drugs act immediately and cause some cytological change.

I'm not quite clear on pp 23, 24, and c (2).

p 23. Ryan suggested the linkage discussion - I may be able to talk him out of it. The main point in Table III though it is not stressed sufficiently is that the various recombination classes, as well as the prototrophs, follow the linear relationship. The variance between experiments is something unexplained and an added feature. Control platings of lt-- on L/ or on T/ give very few single reversions (with triply washed agar 3 EtOH washes) - zero to 20/10 to the 9th cells, average of about 3. This is negligible in comparison to the numbers of protos.

p 24. The mating type differentiation experiment is poor stuff - I'm perfectly more than willing to drop it.
general c (2). Should I mention agglutination as a possible explanation for induction (sufficient number of cells must clump fora sufficient length of time before two cells can undergo syngamy) and saturation (interference of cells in a clump preventing all possible pairings)? This is your idea so I'll have to say (J. L... pers comm). I think it highly likely.

Now for the detailed comments I wrote out after the first letter:
title - You're right - "kinetics of" does sound better but I dropped it for some reason after discussion with FJ - probably because most of the paper was on something besides time variables.
place of publishing - Stern has it already - Ryan said send it in immediately (reports of other labs working on it) - it would probably go to you for refereeing. Biometrics would be OK except I'm not a biometrist (as Levene will heartily agree). If I published there I would be followed by it forever.

p. 1. Yes, I should include Tatum and Lederberg, J. B.
p. 2. This is a section demanded by Ryan. It wasn't until your diploid paper that I remember his taking a definite stand (but this may be a trick of my memory). Assuming 10 to the 9th cells on the plate and each cell forming a microcolony of 10 to the 5th cells, anything larger being visible, this gives 10 to the 14 cells (the medium wouldn't support this many, 0.05% glucose gives 6 X 10 to the 8th/ml, 0.5% gives more but not ten times as many - about 4 to 6 because of acid limitation in liquid culture, but if 6 X 10 to the 9th/ml were obtained this is 9 X 10 to the 10th cells per plate) which if the rates are 10 (-7)) and are independent gives 1 (one) prototroph per plate. 10 (6) cells per microcolony gives 10 protos and so forth. Ryan is more worried about what happens in supplemented agar naturally - as you mention (but as my controls don't show but the experiment hasn't been done correctly - should triple layer - cp-- on bottom, protection layer, and lt-- on top using L and T agar). Witkin brought something close to this up at the oral - what about a pleitrophic (see what new terminology does - pleitropic) mutation, suppressor mutation really, which causes prototrophy in a single step? S/N Control platings were done on this - no double reversions found.
p. 3. Yes as regards Escherichia coli.
If mating type or sexual differentiation or both occur than f in the equations must be replaced by separate f's for the two types or sexes but as you say this doesn't change things. I put it in to answer anyone who might raise the problem.
p. 4. Kinetic data is never critical to prove, that is sufficient, an hypothesis in my book. This may be being too conservative, But it is based on a good grouding in visual kinetics where the data is right but the old interpretation is probably wrong - yet the kientic data will describe the old and the new ideas equally well.
re Kann - OK Have you read her thesis? Why she didn't hit the plate data I don't rightly know - probably a combination of:
wrong strain - 175-12 is rotten/ rotten - so is Y161 due to linkage and to inhibition of prototrophs (as shown by reconstruction experiments) mainly
When a cross gives few protos I usually ascribe it to close linkage - so far there have been no anomalous cases in the strains I've tested (several more than are listed in the paper). When, even after long mixing and high concentration few protos and a decrease of protos

occurs, I consider plate inhibition of the protos or syngamic process by the parental cells which seems to be a function of the specific strain (of its biochemistry). This has been born out in several cases by the reconstruction experiments.

agar not washed in boiling EtOH
didn't keep up the experiments long enough and with enough intermediate points and with controlled shaking

The results in liquid are obviously explained by the kinetics equation.

p. 5. and ff. FJ again - he doesn't get math too fast so he wanted the complete development. I agree that it is ~~apee~~ wasting and is actually not used. It may be valuable in a quantitative investigation of the secondary plate phenomenon (which are also equally spread around the plate and not satellites of prototrophs). There are two other derivations as well as the ones given. But a reference to Hinshelwood - NO!!!! FJ would autoclave me alive. I'd like a ringside seat at the meeting of Sir Cyril and Saint Francis - yoicks, gadzooks, have at you, Erin go bragh, Killarny, Luria, and Delbruck! Though this may be your little joke in return for Ryan's little joke about the pregnant experiments.
pp. 9-10. FJ wanted me to rake Stone, Wyss, and Haas (hereabouts known as Stone, Cold, and Dead) over the coals and I had a hard time talking him out of it. If their platings are controlled as to shaking and time of standing then the UV data may be OK. I haven't tried it but penicillin does nothing at low concentration.

p. 12. see above
Of course I don't mean triparentage - the famous melange (wouldn't menage get past the editors) a trois (this happy phrase has had the grad students splitting their sides here - for similar tweakings of the professorial tails look at the elections of the SAB - list of nominations)- rules that out. Rather, put in three possible parentals and look for:

A X B, B X C, A X C - what relative proportions occur at equal A, B, C, concentrations?

p. 14. Yes - extra special - no weighing of complete media on the same balance as DT salts - no cotton fibers in the medium - dust caps over stoppers (fresh distilled water, essentially for is lation of nitrogen source requirements so I'm told by Gerald Seaman, Creighton Univ. asst prof, Woods Hole, very smart chap, nitrogen fixation in Tetrahymena)

pp. 15, 16, 18 Yes, you're right.
p. 23. Picking of colonies and streaking on differential media has been done and the proportion is not statistically significant from p = KN1N2 (as you indicated). The discussion is FJ initiated and required. See above re first letter. second letter.

p. 24 P 2. This has been repeated with same results but I agree that it is weak evidence - I'll try to talk Ryan into dropping it.
p. 30. Yes - Schmoluchowski and Einstein - you already have this tho'. Szilard asked about this. The above method has been worked out for phage infection of coli and an efficiency of 1 found. The calculation assumes only diffusion while rotary motion was actually used as well - but not knowing moments of inertia, deceleration and acceleration of motor when sampling, viscosity, etc. makes it hard to calculate the increase in collision frequency due to shaking (whose object was not to increase rate

of collision but rather to get a steady mixing). This would take an expert rheologist, Warburging the cross with a translatory motion gave bad results - they scattered all over the map. Possibly the violent agitation is too much for the agglutination or clumping.

Table I Right, will include. If amino acids are natural they may be contaminated with sufficient biotin.
Davis - you've seen his MGB and Experientia papers by now.
Table II OK - just so they don't have to take my word for it.
Table III OK - Should the second part really be dropped? Some geneticists would have a difficult time figuring it out (if those kind read the paper anyway). Statistical tests are run on raw data (first part) but could be run on second with propagation of errors by total differential.
Figures 3 - 7 are all 679-680 X Y 24. It says so in the text but maybe it should in the caption.
Figure 8 - t symbol is bad - what about m or q? This figure stumps them all but it's better than two figures.
Figure 13 Right, Kann gets syntrophy when one substrain is greater by 10 (3). Of course if you have 10 (6) y- and 10 (3) x- then maybe syntrophy will give 10 (4) x- but if one starts with 10(6) of each and gets the same number of cell divisions then the increase in x- is hardly detectable. Cutting out protos is something I haven't tried - something like ascospore isolation or even colony picking.

p. 28 OK

general
(a) My impression too but not FJ - 3 thru 7 might be presented in a group in the text:

// The appearance of secondary prototrophs is characterized by the following pheneomena (which have been experimentally verified quantitatively but have not been presented graphically):

(1) The number of protos per plate increases with time and reaches a maximum at about 100 hours.

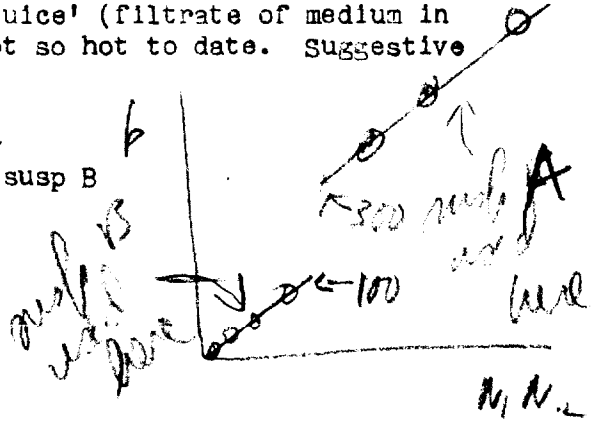
(2) etc. for Graph 4
(3) etc. for Graph 5 //

(b) Yup - the borderline between syngamy and transformation will depend upon the physical methods of 'meiosis' in bacteria - upon the definition of the process. For the time being let's define transformation as genetic trasference without intercellular contact and fusion.

Don't think that I haven't done a lot of microscope squinting - with no results. There are so many funny looking forms that I give up - may be some are undergoing syngamy. Mixtures of two substrains look the same as the unmixed substrain controls but then intratype matings may occur.

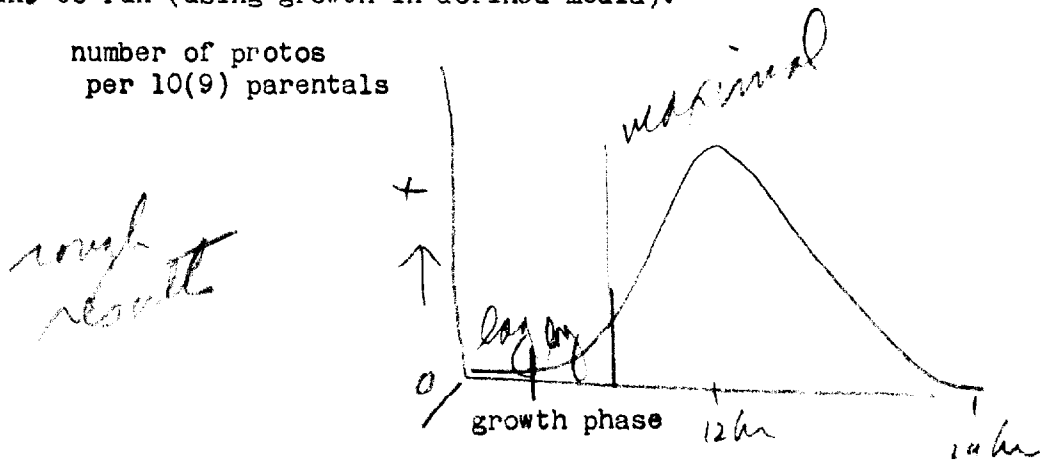
(c) I've had several experiments with 'sex juice' (filtrate of medium in which syngamy has occurred) but results are not so hot to date. Suggestive results are:

(1) put 10(9) cells/ml in saline - susp A
(2) dilute 1:9 equal to 10(8) cells/ml - susp B
(3) run a cross on plates and get:



This doesn't happen in crosses run in liquid and diluted rapidly and plated - so the effect is on the formation of 'zygotes' and not on ability to stick together once fused. In susp A there may be more gamones - no dilution.

Naturally I plan to run (using growth in defined media):



(c - 2) Agglutination! It looks like it in suspensions.
(d) FJ again but I like your idea better.

The news of the conference is trickling in - Kim to Columbia. What's this about the killing curves of diploids being superimposable on the killing curves of haploids?! This suggests:

(1) one hit hypothesis for straight line is wrong - many hits is right (accumulation effect)
(2) Killing is not due to lethal mutations (genic, nuclear) - Kim. What about the centriole, sensitive enzyme systems, etc.

If nuclear killing occurs (induction of lethal mutations) then you should get segregation - recovery of the parental types - is there any?

Bad news at Columbia - all the Public Healths were turned down. Peggy Lieb is badly off - will finish in September and has been accepted by Delbrück for next year if she gets a fellowship. She's pretty sharp and a hard worker - probably the result of an inferiority complex about her sex.

A letter came from FJ - all about the marvelous food (or it should be cuisine) and liquor on the boat. If he keeps up the imbibing and stuffing he'll really look like an Irish leprechaun.

Since I'll be leaving here the 23rd you can reach me at CalTech. With the length of this last missile you may not finish it until you arrive there.

Sincerely,

Ed Lewis